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Resident Wild Birds: A Potential Source for Fluoroquinolone-resistant *Escherichia coli*

Fahim Haque Neloy[1], Md. Liton Rana[1], Sadia Afrin Punom[1] and Md Tanvir Rahman[1]*

[1]*Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.*

Abstract

Antibiotic resistance, commonly known as antimicrobial resistance, is a major global health concern. In Bangladesh, resident wild birds such as the house crow, common myna, and house sparrow can be found near human settlements all year. As a result, these birds could carry fluoroquinolone-resistant *Escherichia coli*. We collected 134 freshly dropped fecal samples for this study. *E. coli* was confirmed using morphological characteristics from culture plates as well as PCR. The Antibiogram of the target isolates was determined by a disk diffusion method and the presence of any resistant genes in the isolates was determined by PCR. About 83% of the common wild birds were found positive for *E. coli* in their fecal samples. In the antibiogram study about 16 to 40% of isolates were found resistant to different fluoroquinolones. Forty-five percent of isolates were found to carry the fluoroquinolone-resistant gene *qnrA*, whereas the *qnrS* gene was absent in all the isolates. All of the isolates examined were found to be resistant to ampicillin and ceftazidime. There are no published data on fluoroquinolone-resistant *E. coli* isolated from common resident wild birds in Bangladesh. Our data imply that house crow, common myna, and house sparrow could be a possible source for the spread of fluoroquinolone-resistant *E. coli* in the environment.

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Introduction

Fluoroquinolones (quinolones) are a class of synthetic broad-spectrum antibiotics that kill bacteria by interfering with DNA replication. Jurado *et al.* (2008) discovered fluoroquinolones as a by-product in the 1960s while researching antimalarial drugs. In the livestock and poultry industries, fluoroquinolones are the most frequently used antibiotics. It inhibits topoisomerase enzymes of bacteria by interfering with the DNA synthesis mechanisms. Fluoroquinolones inhibit topoisomerase II in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria (Hricová *et al.*, 2017). Resistant genes *gyrA, qnrA,* and *qnrS* help bacteria build resistance against fluoroquinolones by enhancing resistance activities in the bacterial DNA (Mahmud *et al.*, 2018).

Antibiotic resistance, commonly known as antimicrobial resistance (AMR) is global public health concern (Karmoker *et al.*, 2003). According to the G20 partners' statement, AMR is a major "growing threat to public health and economic growth". Each year around 700,000 people die all over the world because of AMR (IOM, 2010). Nowadays, antibiotic-resistant bacteria are widely distributed throughout the environmental and clinical samples, and their negative impact has been increasing prominently. Farmers rely heavily on

*Corresponding author e-mail: tanvirahman@bau.edu.bd

antibiotics to combat diseases and infections while raising food-producing animals on an industrial scale and increasing agricultural production. These conditions encourage the selection, transmission, and persistence of antimicrobial-resistant bacteria in the environment, allowing them to cross species boundaries and infect wild birds, country borders, and wild animals, as well as domestic animals and humans (Kleina *et al.*, 2018).

Escherichia coli is a member of the Enterobacteriaceae family. It is a Gram-negative enteric bacteria that can be harmful and resistant to antibiotics. *E. coli* is also zoonotic pathogens (Ievy *et al.*, 2020). The rise of multi-drug resistance (MDR) *E. coli*, notably those resistant to fluoroquinolones, has increased significantly, posing issues for treating infections and restricting doctors' therapeutic options. In Bangladesh, a limited number of research have been done to investigate the incidence of fluoroquinolone-resistant *E. coli* in humans and their genetic features (Akter *et al.* 2011). Many other countries have reported on quinolone-resistant *E. coli* in cattle and other animals (Dues *et al.*, 2015). Mahmud *et al.* (2018) conducted a study to detect fluoroquinolone-resistant *E. coli* in healthy broilers and to determine the presence of the fluoroquinolone-resistant genes *qnrA* and *qnrS*.

Bangladesh has a diverse population of wild birds. Common resident wild birds that live near human settlements, such as the house crow (*corvus splendens*), common myna (*acridotheres tristis*), and house sparrow (*passer domesticus*), can be found throughout the country all year. As a result, these birds could carry a wide range of diseases, including antibiotic-*resistant E. coli*. Pathogens can spread into the environment and affect humans via these carrier birds. However, as stated above, in Bangladesh, there is some evidence of detecting quinolone-resistant *E. coli* from apparently healthy cattle and broilers. According to our literature search, there is no publicly available data on quinolone-resistant *E. coli* discovery in House Crow, Common Myna, and House Sparrows. These birds could be a source of AMR *E. coli*, potentially polluting a health component.

The role of migratory birds in disseminating antibiotic-resistant bacteria has been well-studied recently in Bangladesh (Islam *et al.*, 2021a; Islam *et al.*, 2021b; Karmoker *et al.*, 2023). Like migratory birds and poultry, the common resident wild birds may also have the potential to carry antibiotic-resistant bacteria. However, the role of these birds in disseminating the AMR bacteria and resistant genes is still neglected in the country. Therefore, the present study aimed to focus on detecting fluoroquinolone-resistant *E. coli* from common resident birds in Bangladesh having their potential public health significance.

Materials and Methods

Sampling and Transportation

In this study, a total of 134 freshly dropped fecal samples from house crow (n=42), common myna (n=45), and house sparrow (n=47) were collected from Bangladesh Agricultural University (BAU) campus residential and surrounding areas maintaining sterile conditions. The samples were immediately transported to the microbiology laboratory, maintaining a cool chain at the Department of Microbiology and Hygiene, BAU, for the isolation and characterization of *E. coli*.

Isolation of E. coli

All the collected samples were processed and inoculated on Eosin Methylene Blue (EMB) aerobically at 37 ºC overnight. After overnight incubation, the colonies presenting metallic sheen or light green colors were considered as *E. coli*, which were further confirmed by the morphological characteristics (Gram staining and motility test) and biochemical test as described by Mamun *et al.* (2017) in their study.

Molecular Detection and Characterization of E. coli DNA Extraction Procedures

For the genomic DNA extraction, a pure single colony was taken from the EMB agar plate transferred into 1 mL of nutrient broth, and left for 24 hours incubation at 37 ºC. The genomic DNA was extracted following the boiling and chilling method previously described by Rana *et al.* (2023)

and stored at the temperature below 0 °C for further PCR detection as a PCR template.

Table 1. List of primers used in this study for molecular detection of *E. coli* and resistance genes

Name of target genes	Primer sequences	References
malB	F: GACCTCGGTTTAGTTCACAGA R: CACACGCTGACGCTGACCA	Van *et al.* (2008)
qnrA	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	
qnrS	F: ACGACATTCGTCAACTGCA R: TAAATTGGCACCCTGTAGGC	Robicsek *et al.* (2006)

Molecular Detection of E. coli

Detection of *E. coli* was confirmed by PCR with specific primers as described by Mamun *et al.* (2017). A list of primers to detect *E. coli* and resistant genes used in this study is listed in Table 1.

For the PCR, 20 µL of the reaction mixture for each sample was prepared containing 12 µL of master mix (Takara Bio companies), 1 µL of each primer, 4 µL of nuclease free water, and 4 µL of template DNA. All the components were transferred into a sterile PCR Nanotube and amplified up to 30 cycles in a thermal cycler with an initial denaturation of 5 minutes (min) at 94 ºC ((denaturation for the 30 seconds (s) at 94 ºC, annealing for 30 s at 69 ºC and extension at 72 ºC)) and a final extension for 5 min at 72 ºC. Finally, the amplified genomes were passed through a gel electrophoresis machine with a 100 bp DNA marker at 100 volts for 20 min. After that, the DNA in the gel was stained with 1% ethidium-bromide for 10 min and finally, the gel was photographed under an ultraviolet trans-illuminator (Biometra, Göttingen, Germany). The size of the target amplicon was verified using a 1 kb DNA marker (Promega, Madison, WI, USA).

Antibiotic Susceptibility Test

For a the antibiotic susceptibility test (antibiogram), the Kirby-Bauer disc diffusion method by Hudzicki, J. (2009) was followed. In brief, a 50 mL sterilized petri dish was prepared by Mueller Hinton Agar and kept in an incubator for a sterility test. After the sterility test, 100 µL of pre-enriched isolates maintaining 0.5 McFarland standard spread on the agar plates and some commonly used antibiotic discs (fluoroquinolone, *i.e.*, nrofloxacin, levofloxacin, and ciprofloxacin along with gentamicin, ampicillin, tetracycline, streptomycin, cefotaxime, meropenem, imipenem, fosfomycin, azithromycin, ceftazidime) were placed as per Clinical Laboratory Standards (CLSI), 2020. Twenty randomly selected isolates from each species of birds were subjected to an antibiogram. The antibiogram result was interpreted according to the CLSI standard guidelines (CLSI, 2020), where R is resistant, I is intermediate and S issensitive to a particular antibiotic.

Molecular Detection of Resistance Genes

PCR for the quinolone-resistant gene *qnrA* and *qnrS* gene (Table 1) was done using the primers mentioned above as per Robicsek *et al.* (2016). Randomly selected 20 fluoroquinolone-resistant isolates were subjected to PCR screening, and 9 (45%) were found positive for *qnrA.* None of the isolates were detected positive for *qnrS.*

Results and Discussion

Occurrence of E. coli in Redsident Wild Birds

A total of 134 samples were collected for analysis. Among these 112 (83.54%) samples were found positive for *E. coli.* The overall occurrence of *E. coli* was 83.54%. On a hosted basis the occurrence of *E. coli* was 80.58%, 82.22%, and 87.23% House Crow, Common Myna and House Sparrow, respectively (Table 2). Isolation of *E. coli* was confirmed by culture. Selected isolates were confirmed as *E. coli* by PCR (Figure 1). This study

may be the first report of its kind, which describes the detection of fluoroquinolone-resistant *E. coli* from house crow (*corvus splendens*), common myna (*acridotheres tristis*) and house sparrow (*passer domesticus*) using PCR-based approach.

Table 2. Occurrenceof *E. coli*

Sample	No. of sample	*E. coli* positive	Occurrence (%)
House Crow	42	34	80.95
Common Myna	45	37	82.22
House Sparrow	47	41	87.23
Total	134	112	83.58

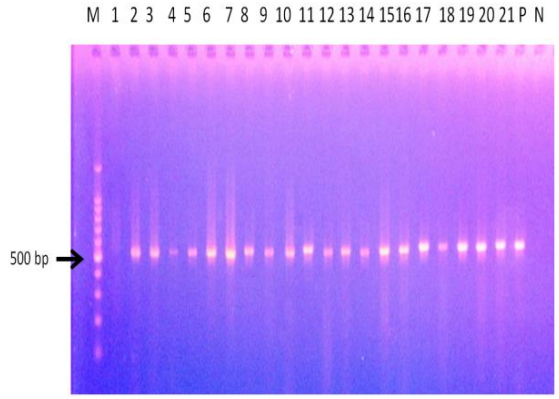

Figure 1. Molecular detection of *E. coli malB* (585 bp) gene. Here, 1 to 21 lanes are representative samples; M stands for marker (DNA ladder); N and P are negative and positive controls, respectively.

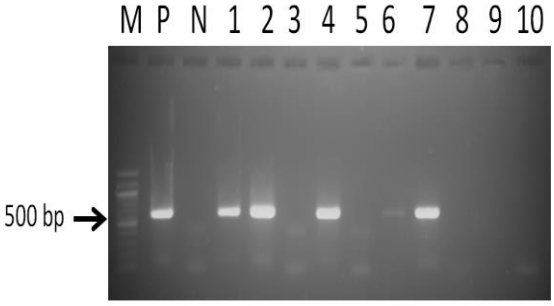

Figure 2. Molecular detection of *E. coli qnrA* (516 bp) gene. Here, M stands for marker (DNA ladder); N and P are negative and positive control, respectively and lanes1 to 7 are representative samples.

Antibiogram and Resistant Gene Detection

A total of 60 randomly selected isolates of *E. coli* (20 from each host) were subjected to antibiogram study by disk diffusion test. The results of the antibiogram are presented in Table 3. About 40%, 35%, and 16.63% of isolates were resistant to fluoroquinolone antibiotics, *e.g.*, enrofloxacin, levofloxacin, and ciprofloxacin respectively. All the isolates were showed resistance against ampicillin and ceftazidine. About 40% of isolates were resistant to tetracycline, 33.33% to streptomycin, 23.30% to azithromycin, and 16.33% to meropenem. No resistance was observed against fosfomycin. Other resistance pattern is presented in Table 3 and Figure 3. Earlier in Bangladesh Mahmud *et al.* (2018) reported an 83.08% occurrence of fluoroquinolone-resistant *E. coil* in healthy broilers. As far our knowledge, no study was performed in Bangladesh, which describes the detection of fluoroquinolone-resistant *E. coli* from common resident wild birds. Belimahdi *et al.* (2023) carried out a study focusing on antibiotic-resistant *E. coli* in crows in Algeria. The highest level of resistance was detected against tetracycline (96.30%) whereas the lowest level was found in aztreonam and ceftazidime (25.92%) followed by cefotaxime, sulfamethoxazole, ciprofloxacin, amoxicillin and imipenem as 22.22%, 11.11%, 7.40%, and 3.70%, respectively. In their study, Mahmud *et al.* (2018) identified 13 (72.22%) positive *E. coli* isolates that had fluoroquinolone-resistant *qnrS* gene from a total of 18 samples. However, they did not mention finding of positive isolates for the *qnrA* gene in their tested isolates. In this study, out of 20 randomly selected fluoroquinolone-resistant isolates 9 (45%) were found positive for *qnrA* in PCR (Figure 2). All these tested isolates were negative for *qnrS*.

Table 3. Resistance pattern of the *E. coli* (n=60) isolates

Name of antibiotics	No. of resistance isolates			Overall resistance (%)
	House Crow (n=20)	Common Myna (n=20)	House Sparrow (n=20)	
Gentamicin	0	4	0	6.60
Ampicillin	20	20	20	100.00
Tetracycline	20	0	4	40.00
Streptomycin	8	6	6	33.33
Cefotaxime	6	6	1	21.66
Meropenem	6	4	0	16.63
Imipenem	10	10	10	50.00
Enrofloxacin	8	8	8	40.00
Levofloxacin	7	4	10	35.00
Ciprofloxacin	6	2	2	16.63
Fosfomycin	0	0	0	0
Azithromycin	8	4	2	23.3.0
Ceftazidime	20	20	20	100.00

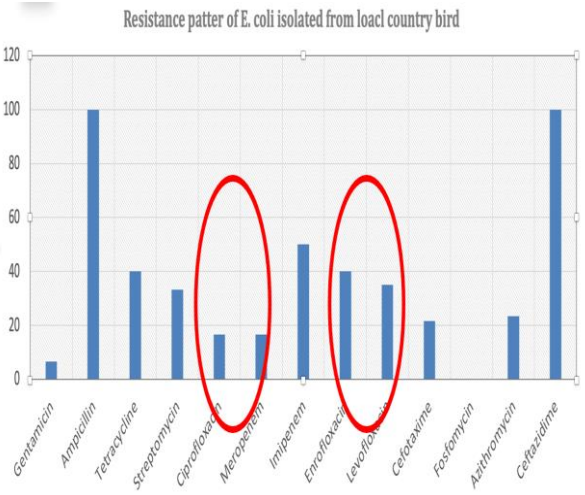

Figure 3. Overall antibiotic resistance pattern of the isolated *E. coli.*

Conclusions

Resident wild birds, namely house crow, common myna and house sparrow are very common in Bangladesh and are found all year round. In this study, many of these birds were found to be positive for *E. coli* in their fecal samples, of which 16 to 40% of isolates were resistant to fluoroquinolones. Fluoroquinolone-resistant gene *qnrA* was detected in a few isolates. From the public health point of view, the presence of fluoroquinolone-resistant *E. coli* in resident wild birds is alarming as these could be a potential source for the spread of fluoroquinolone-resistant *E. coli* to the ecosystem, which will in turn affect humans. We recommend routine monitoring of these brids for pathogen and resistance patterns so that an effective strategy can be adopted to reduce the impact of AMR on one health component.

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Declaration

There is no conflict of interest among the authors.

Authors' Contributions

MTR conceptualized and responsible for supervision, funding acquisition and administration of the project as

well as methodology, software, validation, investigation, formal analyses and draft preparation; FHN, MLR and SAP were responsible for writing and original draft preparation; FHN and SAP were also responsible for reviewing and editing the manuscript. The completed manuscript has been read and approved by all the authors.

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